Association of homocysteine-metabolizing enzyme gene polymorphisms and lipid profiles: an investigation of a gene-environment interaction

Shanqun Jiang^{1,2,*}, Scott A. Venners^{3,*}, Yi-Hsiang Hsu^{4,5}, Justin Weinstock⁶, Suwen Wu¹, Yanfeng Zou⁷, Faming Pan⁷ and Xiping Xu^{2,8}

¹School of Life Sciences, Anhui University, Hefei, China

²Institute of Biomedicine, Anhui Medical University, Hefei, China

³Faculty of Health Sciences, Simon Fraser University, Burnaby, BC, Canada

⁴Institute for Aging Research, HSL and Harvard Medical School, Boston, MA, USA

⁵Molecular and Integrative Physiological Sciences Program, Harvard School of Public Health, Boston, MA, USA

⁶Department of Statistics, University of Virginia, Charlottesville, VA, USA

⁷Department of Epidemiology and Biostatistics, School of Public Health, Anhui Medical University, Hefei, China

⁸Division of Epidemiology and Biostatistics, University of Illinois at Chicago School of Public Health, Chicago, IL, USA

*These authors contributed equally to this work

Correspondence to: Shanqun Jiang, **email:** shanqunjiang2014@163.com

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ABSTRACT

This cross-sectional study investigated the gene-environment interactions between the MTHFR and MTR polymorphisms and gender with regard to lipid profiles in a general Chinese population. We recruited 2,124 individuals from Anhui Province, China. The MTHFR (C677T and A1298C) and MTR (A2756G) polymorphisms were genotyped, and serum lipid levels, including TC, TG, LDL-C, and HDL-C, were determined. Our results showed that male MTR AG + GG genotype carriers had significantly lower serum TC (adjusted $\beta \pm [SE]$: -0.25 ± 0.10 mmol/L; $P = 0.0159$), LDL-C (adjusted $\beta \pm [SE]$: -0.24 ± 0.09 mmol/L; $P = 0.0049$), and HDL-C (adjusted $\beta \pm [SE]$: -0.09 ± 0.03 mmol/L; $P = 0.0055$) levels than the AA carriers. Male MTHFR677TT genotype carriers had significantly higher serum TC (adjusted $\beta \pm [SE]$: 0.28 ± 0.13 mmol/L; $P = 0.0287$) and LDL-C (adjusted $\beta \pm [SE]$: 0.26 ± 0.13 mmol/L; $P = 0.0485$) levels than CC + CT carriers. In subsequent analyses, there was a significant interaction between the MTR AG+GG genotypes and gender in association with TC, LDL-C and TG levels ($P = 0.0378$, 0.0054 and 0.0183 for interaction, respectively). The interaction term for the MTHFR 677TT genotype and gender was also significant for TC and LDL-C levels ($P = 0.0147$ and 0.0243 for interaction, respectively). Further haplotype analysis showed that there also were significant interactions between gender and hap2 (MTHFR 677C/1298A) on TC ($P = 0.009$) and LDL-C ($P = 0.013$). We suggest that the negative effects of the MTR and MTHFR genotypes on serum lipids are based on certain gene-environment interactions in the Chinese general population.

INTRODUCTION

Meta-analysis has shown that an increase of 5 μ mol/L in plasma homocysteine (Hcy) levels enhances the lifetime risk of cardiovascular disease (CVD) 1.6- to 1.8-

fold, similar to the elevation in risk with an increase of 20 mg/dL (0.52 mmol/L) in cholesterol concentration [1]. A growing body of evidence has found that the risk associated with HHcy and hypercholesterolemia combined is greater than the risk associated with one of these risk factors alone

[2]. This suggests that HHcy can accelerate atherosclerosis when associated with elevated plasma lipid levels.

Recently, our China Stroke Primary Prevention Trial (CSPPT) subgroup analysis [3] found that, for participants not receiving folic acid treatment, high total cholesterol (TC) (≥ 200 mg/dL) was an independent predictor of first stroke when compared with low TC. Folic acid supplementation for 4.5 years significantly reduced the risk of first stroke by 31% among hypertensive participants with high TC. In the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study, which enrolled over 9,000 coronary patients with type 2 diabetes, fenofibrate led to just an 11% reduction in coronary events [4] while Hcy levels increased by approximately 35%. Our previous study showed that Simvastatin not only efficiently reduces serum lipid levels, but also causes a significant reduction in Hcy levels in Chinese patients with primary hyperlipidemia [5]. This array of evidence suggests that there is an inherent correlation between HHcy and hypercholesterolemia, and that HHcy can accelerate atherosclerosis dependent on elevated plasma lipids. Unfortunately, the mechanisms linking Hcy and lipid metabolism are not thoroughly known.

Several key enzymes, namely methylenetetrahydrofolate reductase (*MTHFR*) [6] and methionine synthase (*MTR*) [7], are important in Hcy metabolism. *MTHFR* converts 5, 10-methylenetetrahydrofolate into 5-methyltetrahydrofolate, which provides a methyl for Hcy to be catalyzed into methionine by *MTR* [8]. *MTR* requires vitamin B12 (cobalamin) as a coenzyme. When the cobalamin (I) cofactor of *MTR* is oxidized to cobalamin(II), it leads to the inactivation of *MTR*. *MTHFR* C677T (rs1801133, NP_005948.3:p.Ala222Val) is a common variant closely related to CVD risk [6]. The variant creates a thermolabile enzyme with a 50% reduction in activity at 37°C and is associated with a 25% increase in Hcy concentrations [9]. Yilmaz et al. [10] have shown that, by screening the *MTHFR* C677T polymorphism in renal transplant patients, the C allele has a protective effect on blood lipid levels, while the T allele has a harmful effect. As for the *MTHFR* A1298C (rs1801131, NP_005948.3:p.Glu429Ala) polymorphism, there is no clear effect on plasma Hcy levels or serum lipid levels [11]. In hyperlipidemic patients, the *MTR* A2756G (rs1805087, NP_000245.2:p.Asp919Gly) variant was associated with higher levels of TC and LDL-C [12].

Gender differences have been reported as a traditional vascular risk factor for CVD, including hyperlipidemia and HHcy. The third National Health and Nutrition Examination Survey (NHANES III) demonstrated that males have higher serum Hcy concentrations than females [13, 14]. Meanwhile, TG and HDL-C seem to be better predictors of congenital heart disease (CHD) risk than TC or LDL-C in females [15]. Therefore, gender may affect the impact of certain polymorphisms, such as Hcy metabolic enzyme genes on lipid or Hcy levels.

In the present study, we aim to examine the cross-sectional associations between the *MTHFR* C677T, *MTHFR* A1298C, and *MTR* A2756G functional gene polymorphisms and blood lipid levels, as well as whether interactions between these variants exist on the multiplicative scale with gender in a Chinese general population.

RESULTS

General characteristics

In total, 2,124 individuals from two regions, Huoqiu and Yuexi, both in Anhui Province, China, with available genotypes and phenotypes were recruited and analyzed. The genotype distributions of the *MTHFR* C677T and *MTR* A2756G polymorphisms did not deviate from Hardy-Weinberg equilibrium ($\chi^2 = 0.14$, $P = 0.702$ and $\chi^2 = 1.37$, $P = 0.242$, respectively), though the equilibrium did not hold for the *MTHFR* A1298C polymorphism ($\chi^2 = 6.08$, $P = 0.014$). Linkage disequilibrium (LD) plots showed that the *MTHFR* C677T and A1298C single nucleotide polymorphisms (SNPs) were not in complete LD ($D' = 1$, $r^2 = 0.258$). The distributions of participants' age, TC, HDL-C, LDL-C, alanine aminotransferase (ALT), aspartate transaminase (AST), blood urea nitrogen (BUN), total bilirubin (TBIL), creatinine (CR), and albumin (ALB) were significantly different between males and females, as shown in Table 1. There are also significant differences in the prevalence of cigarette smoking, alcohol consumption, occupation and education between males and females

Gender-specific associations between genotypes and serum lipid levels

As shown in Table 2, the levels of TC, LDL-C and HDL-C were significantly different across the three genotypes of the *MTR* A2756G gene in the male sample. Multiple linear regression analysis showed that, compared with the AA genotype carriers, the AG + GG genotype carriers had lower serum TC (adjusted beta $\pm$ SE: -0.25 ± 0.1 mmol/L; $P = 0.0159$), LDL-C (adjusted beta $\pm$ SE: -0.24 ± 0.09 mmol/L; $P = 0.0049$), and HDL-C levels (adjusted beta $\pm$ SE: -0.09 ± 0.03 mmol/L; $P = 0.0055$). After applying the Bonferroni correction, the adjusted P values were still significant. In males, the levels of TC and LDL-C were also different across the three genotypes of the *MTHFR* C677T gene. Multiple linear regression analysis showed that the TT genotype carriers had higher serum TC (adjusted beta $\pm$ SE: 0.28 ± 0.13 mmol/L; $P = 0.0399$) and LDL-C levels (adjusted beta $\pm$ SE: 0.26 ± 0.12 mmol/L; $P = 0.0247$) than the CC + CT genotype carriers. However, after applying the Bonferroni correction, the adjusted P values were no longer significant. There were no significant differences in the levels of TG and HDL-C across the three genotypes of *MTHFR* C677T ($P > 0.05$ for all).

Table 1: Baseline clinical and epidemiologic characteristics of sample grouped by gender

Variables	Male		Female		<i>p</i> -value*
	Mean	SD	Mean	SD	
N	1084		1040		
Age (years)	46.5	9.9	45.2	9.4	0.003
BMIa (kg/m ²)	23.8	3.5	24.2	3.6	0.091
TC (mmol/L)	4.6	0.9	4.7	1.0	0.002
TG (mmol/L)	1.1	0.8	1.1	0.7	0.993
HDL (mmol/L)	1.3	0.3	1.3	0.3	0.002
LDL (mmol/L)	2.8	0.8	2.9	0.8	0.022
ALT(IU/L)	20.5	17.7	15.8	10.4	< 0.001
AST (IU/L)	33.6	19.5	29.6	15.3	< 0.001
BUN (mmol/L)	4.6	1.5	4.1	1.3	< 0.001
TBIL (umol/L)	10.2	4.7	9.5	3.9	< 0.001
CR (umol/L)	64.6	16.7	50.2	11.2	< 0.001
ALB (g/L)	44.6	4.4	45.0	4.8	0.033
	N	%	N	%	
Medication use					
No	955	90.7	899	90.8	0.918
Yes	98	9.3	91	9.2	
Cigarette smoking					
No	477	43.9	984	94.5	< 0.001
Yes	609	56.1	57	5.5	
Alcohol consumption					
No	596	54.9	999	96	< 0.001
Yes	490	45.1	42	4	
Occupation					
Farmer	315	29	505	48.5	< 0.001
Non-farmer	771	71	536	51.5	
Education					
High school or lower	634	58.4	863	82.9	< 0.001
College or higher	452	41.6	178	17.1	
Region					
Huoqiu	649	59.9	606	58.3	0.453
Yuexi	435	40.1	434	41.7	

**t* tests and Pearson's χ^2 tests were applied to the continuous and categorical variables, respectively.

Abbreviations: BMI, body mass index; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate transaminase; BUN, blood urea nitrogen; TBIL, total bilirubin; CR, creatinine; ALB, albumin. aBMI = weight/height². Bold values denoted significant results.

Interactions between gender and genotypes on serum lipid profiles

As shown in Tables 2 and 3, we found significant interactions between genetic variants (*MTR A2756G* and *MTHFR C677T*) and gender in association with serum lipid levels. Table 2 showed that there was a significant

interaction between the *MTR 2756 AG + GG* genotypes and gender in association with serum TC (P interaction = 0.0378), LDL-C (P interaction = 0.0054), and TG levels (P interaction = 0.0183). We also identified the interactions between the *MTHFR 677TT* genotype and gender in association with serum levels of TC (P interaction = 0.0147) and LDL-C (P interaction = 0.0243). However,

Table 2: Association between the MTR A2756G polymorphism and baseline lipid levels by linear regression models

Variables	Gender	MTR			Crude			Adjusted*		
		A2756G	N	Mean $\pm$ SD	Beta	se	p-value	beta	se	p-value
TC	Male	AA	864	4.58 $\pm$ 0.91	Ref.	.	.	Ref.	.	.
		AG	214	4.45 $\pm$ 0.82	-0.13	0.07	0.0529	-0.22	0.11	0.0370
		GG	8	3.89 $\pm$ 0.64	-0.69	0.21	0.0013	-0.50	0.10	< 0.001
		AG+GG	222	4.43 $\pm$ 0.82	-0.16	0.07	0.0188	-0.25	0.10	0.0159
	Female	AA	869	4.67 $\pm$ 0.97	Ref.	.	.	Ref.	.	.
		AG	165	4.71 $\pm$ 1.03	0.04	0.08	0.6019	0.09	0.11	0.4445
		GG	7	4.84 $\pm$ 0.69	0.17	0.22	0.4454	0.20	0.34	0.5434
		AG+GG	172	4.72 $\pm$ 1.02	0.05	0.08	0.5523	0.09	0.11	0.3867
Test of interaction	Female*(AG+GG)				0.21	0.10	0.0459	0.31	0.15	0.0378
LDL-C	Male	AA	864	2.80 $\pm$ 0.80	Ref.	.	.	Ref.	.	.
		AG	214	2.67 $\pm$ 0.71	-0.13	0.06	0.0270	-0.23	0.09	0.0114
		GG	8	2.32 $\pm$ 0.45	-0.48	0.15	0.0017	-0.47	0.17	0.0068
		AG+GG	222	2.66 $\pm$ 0.70	-0.15	0.06	0.0091	-0.24	0.09	0.0049
	Female	AA	869	2.84 $\pm$ 0.83	Ref.	.	.	Ref.	.	.
		AG	165	2.91 $\pm$ 0.82	0.07	0.07	0.3041	0.12	0.10	0.2302
		GG	7	2.88 $\pm$ 0.61	0.04	0.22	0.8597	0.24	0.23	0.3024
		AG+GG	172	2.91 $\pm$ 0.81	0.07	0.07	0.3239	0.13	0.09	0.1800
Test of interaction	Female*(AG+GG)				0.21	0.09	0.0120	0.36	0.13	0.0054
TG	Male	AA	864	1.11 $\pm$ 0.72	Ref.	.	.	Ref.	.	.
		AG	214	1.21 $\pm$ 0.89	0.09	0.07	0.1521	0.11	0.08	0.1562
		GG	8	0.98 $\pm$ 0.74	-0.14	0.25	0.5724	0.17	0.20	0.3873
		AG+GG	222	1.20 $\pm$ 0.89	0.09	0.06	0.1550	0.11	0.08	0.1506
	Female	AA	869	1.15 $\pm$ 0.71	Ref.	.	.	Ref.	.	.
		AG	165	1.04 $\pm$ 0.53	-0.10	0.05	0.0296	-0.11	0.06	0.0503
		GG	7	1.16 $\pm$ 0.77	0.01	0.29	0.9590	-0.07	0.29	0.7975
		AG+GG	172	1.05 $\pm$ 0.54	-0.10	0.05	0.0476	-0.11	0.06	0.0600
Test of interaction	Female*(AG+GG)				-0.19	0.08	0.0183	-0.24	0.10	0.0183
HDL-C	Male	AA	864	1.29 $\pm$ 0.33	Ref.	.	.	Ref.	.	.
		AG	214	1.23 $\pm$ 0.29	-0.06	0.02	0.0145	-0.08	0.03	0.0139
		GG	8	1.13 $\pm$ 0.16	-0.16	0.05	0.0023	-0.16	0.05	0.0007
		AG+GG	222	1.22 $\pm$ 0.29	-0.06	0.02	0.0068	-0.09	0.03	0.0055
	Female	AA	869	1.32 $\pm$ 0.33	Ref.	.	.	Ref.	.	.
		AG	165	1.33 $\pm$ 0.31	0.01	0.03	0.6117	0.00	0.03	0.9987
		GG	7	1.43 $\pm$ 0.34	0.12	0.15	0.4429	0.00	0.11	0.9688
		AG+GG	172	1.33 $\pm$ 0.31	0.02	0.03	0.4831	0.00	0.03	0.9923
Test of interaction	Female*(AG+GG)				0.08	0.03	0.0174	0.08	0.05	0.0685

*Adjusted for age, BMI, medication use, alcohol consumption, cigarette smoking, occupation, education and region.

Abbreviations: TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

Table 3: Association between the MTHFR C677T polymorphism and baseline lipid levels by linear regression models

Variables	Gender	MTHFR			Crude			Adjusted		
		C677T	N	Mean $\pm$ SD	beta	se	p-value	beta	se	p-value
TC	Male	CC	350	4.49 $\pm$ 0.86	Ref.	.	.	Ref.	.	.
		CT	520	4.54 $\pm$ 0.90	0.05	0.06	0.4536	0.05	0.10	0.6032
		TT	216	4.66 $\pm$ 0.93	0.17	0.08	0.0345	0.28	0.13	0.0287
		CC+CT	870	4.52 $\pm$ 0.87	Ref.	.	.	Ref.	.	.
		TT	216	4.66 $\pm$ 0.93	0.14	0.07	0.0498	0.24	0.12	0.0399
	Female	CC	306	4.77 $\pm$ 1.05	0.00	.	.	0.00	.	.
		CT	523	4.65 $\pm$ 0.92	-0.11	0.07	0.1312	-0.09	0.09	0.3535
		TT	212	4.61 $\pm$ 1.02	-0.15	0.10	0.1204	-0.18	0.12	0.1195
		CC+CT	829	4.70 $\pm$ 0.97	Ref.	.	.	Ref.	.	.
		TT	212	4.61 $\pm$ 1.02	-0.08	0.08	0.3252	-0.12	0.10	0.1889
	Test interaction of	Female*TT			-0.22	0.11	0.0374	-0.36	0.15	0.0147
LDL-C	Male	CC	350	2.73 $\pm$ 0.77	Ref.	.	.	Ref.	.	.
		CT	520	2.76 $\pm$ 0.74	0.03	0.05	0.5247	-0.01	0.10	0.9348
		TT	216	2.88 $\pm$ 0.89	0.15	0.07	0.0373	0.26	0.13	0.0485
		CC+CT	870	2.75 $\pm$ 0.75	Ref.	.	.	Ref.	.	.
		TT	216	2.89 $\pm$ 0.87	0.14	0.06	0.0240	0.26	0.12	0.0247
	Female	CC	306	2.93 $\pm$ 0.86	Ref.	.	.	Ref.	.	.
		CT	523	2.82 $\pm$ 0.76	-0.10	0.06	0.0904	-0.08	0.08	0.2915
		TT	212	2.83 $\pm$ 0.93	-0.10	0.09	0.2374	-0.14	0.12	0.2208
		CC+CT	829	2.87 $\pm$ 0.79	Ref.	.	.	Ref.	.	.
		TT	212	2.83 $\pm$ 0.93	-0.04	0.08	0.5958	-0.09	0.10	0.3863
	Test interaction of	Female*TT			-0.18	0.09	0.0490	-0.35	0.15	0.0243
TG	Male	CC	350	1.13 $\pm$ 0.81	Ref.	.	.	Ref.	.	.
		CT	520	1.10 $\pm$ 0.59	-0.04	0.05	0.4573	-0.09	0.08	0.2763
		TT	216	1.21 $\pm$ 0.99	0.07	0.08	0.3451	0.08	0.11	0.4373
		CC+CT	870	1.11 $\pm$ 0.69	Ref.	.	.	Ref.	.	.
		TT	216	1.18 $\pm$ 0.91	0.07	0.06	0.2776	0.14	0.09	0.1155
	Female	CC	306	1.12 $\pm$ 0.60	Ref.	.	.	Ref.	.	.
		CT	523	1.14 $\pm$ 0.71	0.01	0.05	0.7656	-0.07	0.06	0.2642
		TT	212	1.13 $\pm$ 0.73	0.01	0.06	0.8837	0.05	0.10	0.5853
		CC+CT	829	1.13 $\pm$ 0.66	Ref.	.	.	Ref.	.	.
		TT	212	1.13 $\pm$ 0.73	0.00	0.05	0.9368	0.10	0.09	0.3009
	Test interaction of	Female*TT			-0.06	0.08	0.4381	-0.04	0.13	0.7399
HDL-C	Male	CC	350	1.27 $\pm$ 0.32	Ref.	.	.	Ref.	.	.
		CT	520	1.28 $\pm$ 0.32	0.01	0.02	0.6089	0.04	0.03	0.2039
		TT	216	1.26 $\pm$ 0.31	-0.01	0.03	0.7801	0.01	0.04	0.8854
		CC+CT	870	1.28 $\pm$ 0.32	Ref.	.	.	Ref.	.	.
		TT	216	1.26 $\pm$ 0.31	-0.02	0.02	0.4584	-0.02	0.04	0.5326
	Test interaction of	Female*TT			-0.06	0.08	0.4381	-0.04	0.13	0.7399

	Female	CC	306	1.33 ± 0.31	Ref.	.	.	Ref.	.	.
		CT	523	1.32 ± 0.34	−0.01	0.02	0.6067	0.03	0.03	0.3198
		TT	212	1.30 ± 0.32	−0.03	0.03	0.2743	0.00	0.04	0.9588
		CC+CT	829	1.32 ± 0.33	Ref.	.	.	Ref.	.	.
		TT	212	1.30 ± 0.32	−0.02	0.03	0.3605	−0.02	0.03	0.6200
Test of interaction	Female*TT				−0.01	0.03	0.8743	0.00	0.05	0.9896

*Adjusted for age, BMI, medication use, alcohol consumption, cigarette smoking, occupation, education and region.

Abbreviations: TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

after adjusting for conducting multiple tests using the Bonferroni correction, most of these interactions were no longer significant. However, basically there were no significant results observed in Table 4.

Haplotype analyses

Three haplotypes with frequency > 0.01 (T-A, C-A, and C-C) were observed for SNP rs1801133-rs1801131 (Table 5). T-A was the most common haplotype observed in 44% of our study population. Haplotype-specific association tests with additive haplotype effects found that, in males, compared to the most common haplotype T-A, patients carrying the haplotype C-C had significantly lower levels in TC ($P = 0.027$). However, in females, patients carrying the haplotype C-A had significantly higher levels in TC ($P = 0.029$). The interaction term of gender*hap2 (haplotype C-A) was significant for TC ($P = 0.009$) and LDL-C ($P = 0.013$), and gender*hap3 (haplotype C-C) was marginally significant for TC and LDL-C.

DISCUSSION

Our previous studies [16, 17] examined the effects of Hcy metabolic gene polymorphisms and their interactions with environmental factors on serum lipid levels, leading us to conclude that both the *MTHFR* and *MTRR* gene polymorphisms could be important genetic determinants of serum lipid levels dependent on environmental factors, including Hcy, gender, alcohol consumption, and smoking status, in patients with hypertension. In order to confirm and better generalize these findings, we further investigated the effects of the Hcy metabolic gene polymorphisms and their interactions with gender on serum lipid levels in this study's relatively larger general population from distinct geographic regions. Our results show that the *MTR* and *MTHFR* genotypes constitute risk factors for elevated lipid levels conditional on gender. The *MTHFR* C677T polymorphism, a common variant, is located in the catalytic domain of the enzyme. It can cause an alanine to valine substitution at position 222 of the enzyme, creating a thermolabile enzyme [6], and has been related to elevated plasma Hcy concentration [9]. Our previous [16, 17] and present studies consistently

demonstrated that individuals with the 677TT genotype had significantly higher TC and LDL-C levels than those with the 677CC + CT genotypes in a Chinese hypertensive or general population. Yilmaz et al. [10] and Zhang et al. [18] have reported that carriers of the 677T allele have significantly higher TC and LDL-C than 677T allele non-carriers. Several other studies also confirmed that *MTHFR* C677T is a key genetic determinant of lipid profiles. For example, evidence that the *MTHFR* gene polymorphism is an important independent contributor to TC and HDL-C was found by Kawamoto et al. [19], and Pitsavos et al. reported that TC and TG are statistically different across *MTHFR* genotypes [20]. Another functional polymorphism in the *MTR* gene is A2756G, located at position 919 of the protein and resulting in a substitution of glycine for aspartic acid [21]. Our present study showed that, compared with AA genotype carriers, the male individuals carrying the AG+GG genotype had significantly lower serum TC ($P = 0.0159$), LDL-C ($P = 0.0049$), and HDL-C levels ($P = 0.0055$) in the general population. On the contrary, in a relatively small sample size of hyperlipidemic patients, *MTR* 2756AG+GG carriers had higher TC and LDL-C levels than 2756AA carriers [12].

Elevated Hcy level has a direct toxic effect on atherogenesis. The underlying mechanisms by which HHcy promotes atherogenesis may be stimulation of vascular smooth muscle cell proliferation [22], inhibition of endothelial cell growth and post-injury reendothelialization, impairment of endothelial relaxation, and accelerated neointimal formation [23–25]. Additionally, in an animal model, HHcy inhibited reverse cholesterol transport by reducing circulating HDL-C levels, via inhibition of apoA-I protein synthesis, and enhanced HDL-C clearance [26]. Therefore, it seems that the liver may be a major organ involved in regulating Hcy and cholesterol homeostasis [27].

Several studies have shown that the male gender is a significant predictor of carotid atherosclerosis. The influence of inflammation on survival was reported to be more pronounced in males than females [28]. Interestingly, estrogen has significant antioxidant properties [29] and limits the inflammatory response to injury by modulating the expression of cellular adhesion molecules from the

Table 4: Association between the MTHFR A1298C polymorphism and baseline lipid levels by linear regression models

Variables	Gender	MTHFR			Crude			Adjusted*		
		A1298C	N	Mean $\pm$ SD	beta	se	p-value	beta	se	p-value
TC	Male	AA	739	4.59 $\pm$ 0.88	Ref.	.	.	Ref.	.	.
		AC	324	4.46 $\pm$ 0.92	-0.13	0.07	0.0439	-0.25	0.10	0.0122
		CC	23	4.45 $\pm$ 0.88	-0.13	0.18	0.4557	0.19	0.24	0.4227
	Female	AA	740	4.68 $\pm$ 0.98	Ref.	.	.	Ref.	.	.
		AC	284	4.68 $\pm$ 0.99	0.00	0.07	0.9885	-0.03	0.08	0.7042
		CC	17	4.43 $\pm$ 0.75	-0.25	0.18	0.1674	-0.45	0.22	0.0450
LDL-C	Male	AA	739	2.81 $\pm$ 0.78	Ref.	.	.	Ref.	.	.
		AC	324	2.70 $\pm$ 0.80	-0.11	0.06	0.0584	-0.19	0.10	0.0552
		CC	23	2.71 $\pm$ 0.65	-0.09	0.13	0.4892	0.11	0.14	0.4150
	Female	AA	740	2.86 $\pm$ 0.83	Ref.	.	.	Ref.	.	.
		AC	284	2.86 $\pm$ 0.84	0.00	0.06	0.9720	0.00	0.07	0.9843
		CC	17	2.71 $\pm$ 0.52	-0.15	0.13	0.2356	-0.17	0.19	0.3589
TG	Male	AA	739	1.15 $\pm$ 0.81	Ref.	.	.	Ref.	.	.
		AC	324	1.09 $\pm$ 0.64	-0.06	0.05	0.2195	0.00	0.07	0.9843
		CC	23	0.97 $\pm$ 0.32	-0.18	0.07	0.0134	0.01	0.16	0.9608
	Female	AA	740	1.13 $\pm$ 0.64	Ref.	.	.	Ref.	.	.
		AC	284	1.14 $\pm$ 0.80	0.01	0.06	0.8260	-0.05	0.06	0.3795
		CC	17	0.90 $\pm$ 0.34	-0.23	0.08	0.0066	-0.28	0.07	< 0.001
HDL-C	Male	AA	739	1.27 $\pm$ 0.31	Ref.	.	.	Ref.	.	.
		AC	324	1.28 $\pm$ 0.34	0.01	0.02	0.6523	-0.03	0.03	0.2717
		CC	23	1.30 $\pm$ 0.34	0.03	0.07	0.6335	0.06	0.13	0.6323
	Female	AA	740	1.32 $\pm$ 0.33	Ref.	.	.	Ref.	.	.
		AC	284	1.31 $\pm$ 0.32	-0.01	0.02	0.7522	-0.02	0.03	0.5904
		CC	17	1.32 $\pm$ 0.27	0.00	0.06	0.9680	-0.16	0.05	0.0033

*Adjusted for age, BMI, medication use, alcohol consumption, cigarette smoking, occupation, education and region.

Abbreviations: TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol

endothelium. This suggests that gender may affect the impact of Hcy metabolic gene polymorphisms on blood lipids.

Indeed, in our present study, there was a significant interaction on the multiplicative scale between the *MTR* AG + GG genotypes and gender in association with TC, LDL-C and TG levels ($P = 0.0378$, 0.0054 and 0.0183 for interaction, respectively). The interaction term for the *MTHFR* 677TT genotype and gender was also significant for TC and LDL-C levels ($P = 0.0147$ and 0.0243 for interaction, respectively). Similar to our findings, a previous study [30]. found interactions on the multiplicative scale between gender and Hcy/lipid-related SNPs on log-transformed plasma Hcy levels. Specifically, the significant interactions included gender and *MTHFR*, gender and *CRBP2*, and gender and *SCARB1* on Hcy levels. It has been established that the *MTHFR* CT or TT genotypes lead to reduced enzyme activity of *MTHFR*

[7]. and are associated with high plasma Hcy when folate intake or nutritional status is low [31]. Unlike the *MTHFR* 677 TT genotype, the *MTHFR* 1298 CC genotype alone has not been associated with changes in plasma Hcy or plasma folate [32]. Our results also proved that the *MTHFR* 1298 CC genotype alone does not contribute to serum lipid levels.

Similarly, many of the studies that have investigated *SCARB1* SNPs have shown different effects in males and females, suggesting that sex hormones or other factors related to gender may play a mediating role [33]. Recently, in Caucasian women, the *SCARB1* SNP rs838893 and estradiol interaction was strongest in association with HDL-C, TG, and the TG/HDL-C ratio. Therefore, the specific physiological characteristics of females may explain the gender-related differences in SNP genotypes that were observed to be associated with lipid profiles, although the underlying mechanism has yet to be elucidated. To the

Table 5: Haplotype analyses of MTHFR gene and their interaction with gender in lipid profiles in general population

Haplotype		N	Hap-Freq	TC*		LDL*	
Variables	rs1801133/rs1801131			β(se)	Pval	β(se)	Pval
Haplotype GLM Analysis							
Male							
Hap1	T- -A	1912	0.45	0		0	
Hap2	C- -A	1656	0.39	−0.13 (0.07)	0.063	−0.09 (0.07)	0.160
Hap3	C- -C	680	0.16	−0.21 (0.09)	0.027	−0.15 (0.09)	0.101
Female							
Hap1	T- -A	1912	0.45	0		0	
Hap2	C- -A	1656	0.39	0.13 (0.06)	0.029	0.10 (0.06)	0.067
Hap3	C- -C	680	0.16	−0.008 (0.08)	0.918	0.03 (0.07)	0.632
Haplotype and Gender Interaction							
Gender*hap2				0.16 (0.06)	0.009	0.13 (0.05)	0.013
Gender*hap3				0.15 (0.08)	0.071	0.14 (0.07)	0.057

* Haplo GLM analyses was adjusted for age, BMI, region, smoking and alcohol status

best of our knowledge, our study is the first to show that the disadvantageous effects of the *MTR* and *MTHFR* genotypes on serum lipids are generalized to the general population based on certain gene-gender interactions.

As for Hcy levels, according to the third National Health and Nutrition Examination Survey (NHANES III), males have higher serum Hcy concentrations than females at the age of 10 years, during adolescence [13], and throughout adulthood [14]. Among a cohort of young adults, individuals at the highest risk for HHcy were smokers across all female subgroups and those with the *MTHFR* 677TT genotype across all male subgroups, thus suggesting different determinants of Hcy levels between males and females [34]. Furthermore, plasma Hcy levels were associated with arterial stiffness only in males [35]. Circulating Hcy levels were also significantly higher in males with CHD than in males without CHD, whereas no difference was observed in Hcy levels in females with regard to CHD [36]. As for lipid profiles, TG and HDL-C seem to be better predictors of CHD risk than TC or LDL-C in females [15]. Males and postmenopausal females had higher levels of serum TC and TG subclasses compared with premenopausal females [37]. Males seem to have higher levels of postprandial TG than females [38]. All of this aforementioned evidence supports our finding that there exists an interactive effect between gender and certain Hcy metabolic gene polymorphisms on blood lipid levels.

In the last few years, the hypothesis that Hcy metabolism can affect blood lipid levels has gained further attention. Therefore, one might speculate that lowering Hcy by means of B-vitamins can affect blood lipids or lowering lipids by statins can influence Hcy levels. Olthof et al. discovered that folic acid supplementation for short-term treatment lowered Hcy by approximately 21%, but changes in TC, HDL-C, LDL-C and TG were not significant at

the end of the treatment [39]. Another study found that supplementation of folic acid for four weeks improved endothelial function without significant changes to plasma lipids [40]. In a relatively long-term (three month) placebo-controlled study on peritoneal dialysis patients, folic acid supplementation led to a 33% reduction in Hcy and significantly decreased levels of TC, LDL-C, and TG [41].

The strength of our study is a relatively large sample size with a high statistical power to increase the precision of our estimates of the association between *MTR* and *MTHFR* genotypes and lipid profiles. Additionally, our findings have also been replicated in the Chinese general population. However, the major limitations of our study are its cross-sectional design and the lack of measurements for plasma Hcy levels and *MTRR* genotyping. Although we cannot conclude a causal relationship between plasma Hcy levels and lipid profiles, our single loci, haplotype and interaction analyses consistently supported that the negative effects of the *MTR* and *MTHFR* genotypes on serum lipids are based on certain gene-environment interactions in the Chinese general population. Furthermore, we only assessed Chinese participants in the present study, and further studies in other ethnic groups are needed to demonstrate whether our findings can be generalized to other populations. Therefore, large, prospective trials involving populations from multiple ethnic groups are needed to better assess the effects of the *MTR* and *MTHFR* polymorphisms on cholesterol homeostasis.

MATERIALS AND METHODS

study population

In the present study, a total of 2,124 participants were enrolled from Huoqiu and Yuexi, two communities

in Anhui Province, China. There were 1,084 males (51%) and 1,040 females (49%). The study participants were enrolled after responding to study announcements in their specific geographic areas when attending health check-up examinations that were commissioned by their local governments. Enrollment criteria included: (1) age 23 to 79 years, and (2) not taking medications known to affect serum lipid levels (lipid-lowering drugs such as statins or fibrates, beta-blockers, diuretics, or hormones) for four weeks before the study. Participants with a history of any of the following conditions were excluded: secondary hypertension, pregnancy, hypercalcemia, chronic CVD, chronic cerebrovascular disease, all kinds of cancers, chronic liver or renal diseases, or body mass index (BMI) above 33 kg/m². The study protocol was approved by the ethics committee of the Institute of Biomedicine at Anhui Medical University. The purpose and procedures of the study were carefully explained to all the participants, and written informed consent was obtained.

<p>Laboratory</p> <p>Venous blood samples were drawn and collected in ethylenediaminetetraacetic acid tubes between 8:00 AM to 10:00 AM after a fourteen-hour fast. In our analytical center, serum lipid parameters were measured by reflective photometry using an automatic biochemistry analyzer, but LDL-C was calculated by Friedewald's equation. The automatic biochemistry analyzer based on spectrophotometric principle is one of the necessary instruments for clinical diagnostics in hospital. TC and TG were determined enzymatically with the cholesterol oxidase/p-aminophenazone method (CHOD-PAP) and the glycerophosphate oxidase/p-aminophenazone method (GPO-PAP), respectively. HDL-C determination was by phosphotungstic acid and magnesium chloride precipitation. Blood samples were drawn and collected in EDTA tubes and then centrifuged at 3000 rpm for 10 minutes to obtain the serum. In order to ensure optimum operation, the automatic biochemistry analyzer was warmed up 10 minutes after turning on. Serum samples were placed in a rack of test tubes, which was rotated through a stepper motor for positioning of blood samples through the measurement chamber of the analyzer. For example, serum cholesterol was estimated by mixing a 0.03 ml serum sample with 3 ml of matching working reagent, and the absorbance of the assay mixture was measured by a spectrophotometer at 546 nm, against distilled water as a blank. Similarly, different working reagents for all biochemical indexes were used for their estimation. The intra- and inter-assay coefficients of variation were less than 5% for all assays performed.</p>	<p>Determinations</p>
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Genotyping of the *MTHFR* and *MTR* Polymorphisms TaqMan allelic discrimination technique was used for detecting *MTHFR* C677T (Ala222Val), *MTHFR* A1298C (Glu429Ala), and *MTR* A2756G (Asp919Gly) genotypes in our central laboratory. Universal reaction conditions for genotyping were as follows: 4 ng dried DNA, 0.08 mL 40

assay locus-specific probe, and 2.0 mL TaqMan universal polymerase chain reaction (PCR) master mix made to a final volume of 4 mL with 1.92 mL of sterile water. The PCR cycle conditions consisted of an initial denaturation at 95°C for ten minutes, followed by fifty cycles of 92°C for fifteen seconds and 60°C for one minute. All sample sets genotyped for each SNP in our present study had overall call rates of 95%, after excluding samples that consistently failed. Concordance of 100% was repeated for all samples' quality control.

Statistical analysis

The Chinese version of Epidata 3.1 was used for database design, data entry, and data check. Mean $\pm$ standard deviation were calculated for continuous variables. One-way analysis of variance and *t*-tests were used to compare the mean differences for continuous variables. The chi-square test was used for categorical variables. Allele frequency was determined via direct counting, and the standard goodness-of-fit test was used to test Hardy-Weinberg equilibrium. TC, TG, HDL-C and LDL-C approximately followed the normal distribution. A multivariate linear regression model was used to evaluate the effect of *MTHFR* and *MTR* polymorphisms on the baseline TC, TG, HDL-C, and LDL-C levels before and after adjusting for possible confounding factors, including age, BMI, medication use, alcohol consumption, cigarette smoking, occupation, education and region. The interactions on the multiplicative scale of the *MTHFR* and *MTR* genetic variants with gender in association with serum lipid levels were tested by adding a product term to the linear models. Haplotypes of SNPs rs1801133 (C677T), and rs1801131 (A1298C) in the *MTHFR* gene were constructed by expectation-maximization algorithm and implemented in R Haplo.stats package. Bonferroni correction was applied for multiple tests. Differences were considered to be significant at *P* values less than 0.05 divided by the number of tests. All statistical analyses were carried out using the SAS software (Release 8.0; SAS Institute, Cary, NC).

CONCLUSIONS

In conclusions, the *MTR* and *MTHFR* genotypes constitute risk factors for lipids conditional on gender. We suggest that the disadvantageous effects of the *MTR* and *MTHFR* genotypes on serum lipids are based on certain gene-environment interactions in the Chinese general population.

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Author contributions

Shanqun Jiang and Scott A. Venners wrote the article; Shanqun Jiang, Scott A. Venners, Yi-Hsiang Hsu, Faming Pan and Xiping Xu designed the research; Shanqun Jiang, Scott A. Venners, Yi-Hsiang Hsu, Justin Weinstock, Suwen Wu, Yanfeng Zou, Faming Pan, and Xiping Xu performed the research; Shanqun Jiang and Scott A. Venners analyzed the data.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest interests.

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